

Detection and molecular characterization of canine circovirus circulating in northeastern China during 2014–2016

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Table S1 Primers used in this study

Target virus	Primer name	Primer sequences (5'-3')	Target gene	Amplicon size (bp)	Annealing temperature (°C)
CanineCV	CanineCV11F	5' TGTATAACTACTGAAAGATAA 3'		1129	49
	CanineCV11L	5' GGTGCCGGGTAATACTAT 3'			
	CanineCVgF	5' GCGAGAGCTGCTCCTTAT 3'		1756	49
	CanineCVgL	5'CGGCCACAGATTAAGTACTT 3'			
	CanineCVF	5' AGGAGGATGCCGTGAA 3'		562	49
	CanineCVL	5' CCATAGAAGTCATCCAGAAT 3'			
	CanineCV1-FW	5' CGTATCCGGAGACCTACGTCA 3'		2040	55
	CanineCV1-REV	5'TTCGTCTCTTTCAGTTTCTCTACAG 3'			
CDV	CDV1	ACAGGATTGCTGAGGACCTAT	Nucleoprotein	287	59
	CDV2	CAAGATAACCATGTACGGTGC			
CPV-2	Hfor	CAGGTGATGAATTTGCTACA		611	49
	Hrev	CATTTGGATAAACTGGTGGT			
coronaviruses	IN-2deg	5'-GGGDTGGGAYTAYCCHAARTGYGA-3'	polymerase	452	49
	IN-4deg	5'-TARCAVACAACISYRTCRTCA-3'			
caliciviruses	P290d	GATTACTCCASSTGGGAYTCMAC	RdRp	319	48
	P289d	TGACGATTTTCATCATCMCCRTA			
astrovirus	MA2	GGCTTTACCCACATICCAAA	Polyprotein	387	55
	MA4	TGGACCCGCTATGATGGCACIAT			
rotaviruses	Con 2	ATTTCCGACCATTTATAACC	VP4	876	50
	Con 3	TGGCTTCGCCATTTTATAGACA			
	S9-as	ACTTGCCACCAYYTYTTCCAATT	VP7	400	55
	S9-csv	ATGAATGGTTATGYAAAYCCDATGGA			

Table S2 Reference strains for Circovirus used in the paper

Reference strains or other circovirus	GenBank Accession No.	Source	Collection date
XF16	MF797786	China	Apr-2016
K1	MK731982	China	Apr-2016
C24	MK731981	China	Apr-2016
C85	MK944080	China	Apr-2016
C79	MK944079	China	Apr-2016
CanineCV 214	JQ821392	USA	2011
CanineCV Ha13	KF887949	Germany	22-Jul-2013
CanineCV JZ98/2014	KT946839	China	2014
CanineCV CD17/2016	MG266899	China	31-Oct-2016
CanineCV PE8575/1-13	KT734823	Italy	Jul-2013
CanineCV TE7482-13	KT734822	Italy	Apr-2013
CanineCV TE6685/1-13	KT734821	Italy	Mar-2013
CanineCV AZ663/1-13	KT734820	Italy	Feb-2013
CanineCV AZ5586-13	KT734819	Italy	Nov-2013
CanineCV AZ5212/1-14	KT734817	Italy	Oct-2014
CanineCV AZ4438-13	KT734816	Italy	Sep-2013
CanineCV AZ4133/1-13	KT734815	Italy	Jul-2013
CanineCV TE4016-13	KT734814	Italy	Feb-2013
CanineCV AZ2972-13	KT734813	Italy	May-2013
CanineCV CB6293/1-14	KT734812	Italy	Nov-2014
CanineCV FUBerlin-JRS	KT283604	Germany	Jun-2014
CanineCV Bari / 411-13	KJ530972	Italy	Jun-2013
CanineCV UCD1-1698	KC241982	USA	Oct-2011

CanineCV UCD2-32162	KC241984	USA	Oct-2011
CanineCV UCD3-478	KC241983	USA	Oct-2011
CanineCV 204	MG279141	China	2017
CanineCV 205	MG279140	China	2017
CanineCV 384	MG279139	China	2017
CanineCV 388	MG279138	China	2017
CanineCV 390	MG279137	China	2017
CanineCV 391	MG279136	China	2017
CanineCV 201	MG279135	China	2017
CanineCV 394	MG279134	China	2017
CanineCV 395	MG279133	China	2017
CanineCV 202	MG279131	China	2017
CanineCV 177	MG279130	China	2017
CanineCV 178	MG279129	China	2017
CanineCV 179	MG279128	China	2017
CanineCV 180	MG279127	China	2017
CanineCV 181	MG279126	China	2017
CanineCV 182	MG279125	China	2017
CanineCV 183	MG279124	China	2017
CanineCV 185	MG279123	China	2017
CanineCV 176	MG279122	China	2017
CanineCV 186	MG279121	China	2017
CanineCV 198	MG279120	China	2017
CanineCV 199	MG279119	China	2017
CanineCV 102	MG279118	China	2017
CanineCV OH19098-1	MF457592	USA	21-Aug-2015

CanineCV 398	MG279132	China	
UBA-Baires	MK033608	Argentina	Mar-2016
WM72	KY388503	China	2015
WM74	KY388502	China	2015
WM76	KY388501	China	2015
WM77	KY388500	China	2015
WM79	KY388499	China	2015
WM83	KY388498	China	2015
WM84	KY388497	China	2015
XXT242	KY388496	China	2015
XXT243	KY388495	China	2015
YL11	KY388494	China	2016
WM62	KY388493	China	2015
WM66	KY388492	China	2015
WM63	KY388491	China	2015
JZ82	KY388490	China	2014
JZ85	KY388489	China	2014
LA128	KY388488	China	2014
LA237	KY388487	China	2014
LA280	KY388486	China	2014
WM46	KY388485	China	2015
WM48	KY388484	China	2015
WM60	KY388483	China	2015
GL51	KY388482	China	2015
JZ50	KY388481	China	2014
GL33	KY388480	China	2015

Table S3 Individual infectious agents in the real-time PCR canine diarrhea panel of dogs

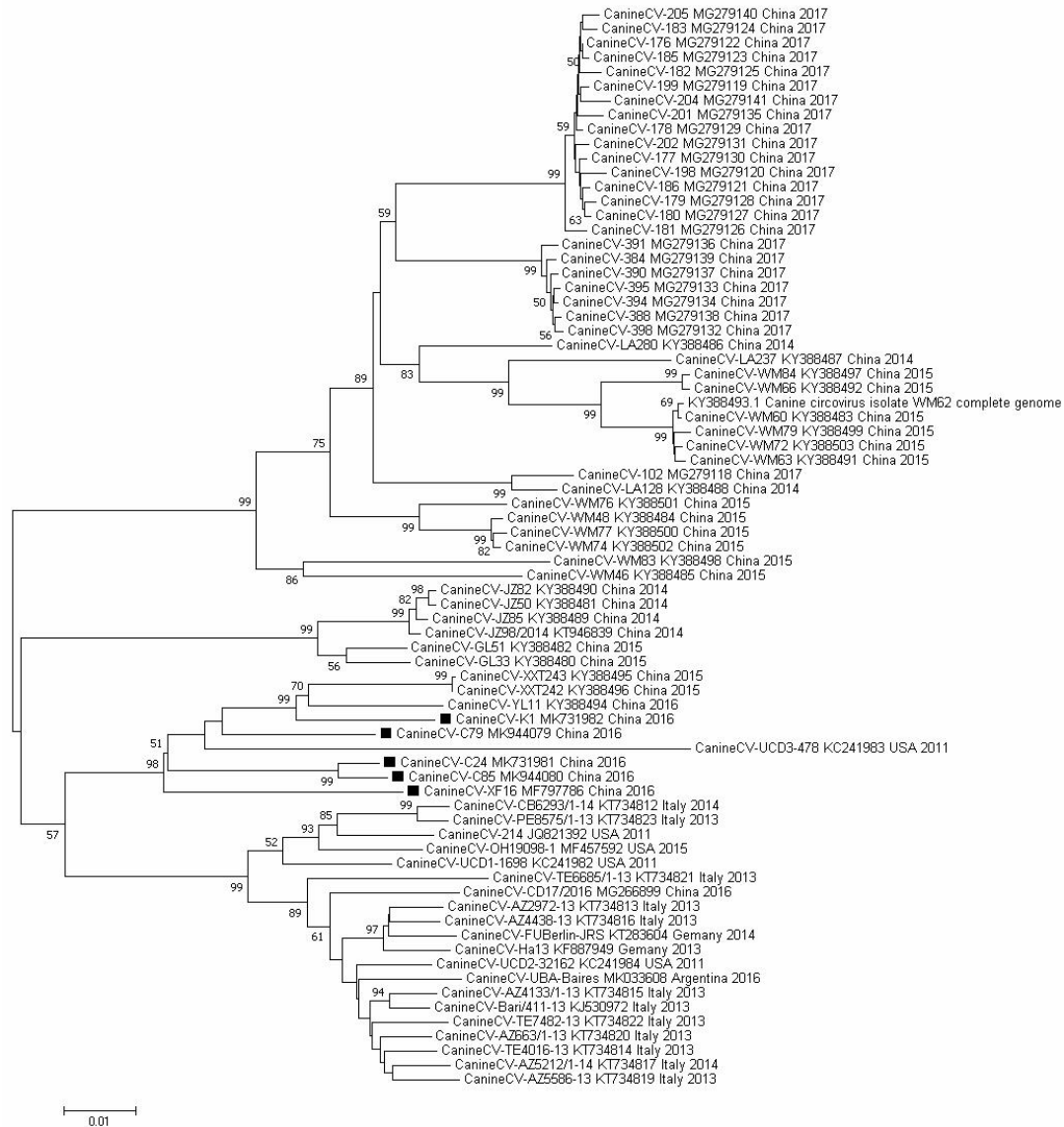
Pathogen	Dogs				P value of Diarrheic and Control	
	Diarrheic (96) n	Healthy (%) n=145				
	(%)	Healthy always (%) n=10	Diarrheic in recent half a year (n=21) (%)	Unknown situation in recent half a year(n=14) (%)		Total (%) n=45
CPV-2*	51/96(53.1)	0/10(0)	0/21(0)	0/14(0)	0/45 (0)	<0.001
CCV*	15/96(15.6)	0/10(0)	2/21(9.5)	1/14(7.1)	3/45 (6.7)	0.009
CCoV	0/96(0)	0/10(0)	0/21(0)	0/14(0)	0/45 (0)	-
rotaviruses	0/96(0)	0/10(0)	0/21(0)	0/14(0)	0/45 (0)	-
caliciviruses	5/96(5.2)	0/10(0)	1/21(4.8)	0/14(0)	1/45 (2.2)	0.146
astroviruses	0/96(0)	0/10(0)	0/21(0)	0/14(0)	0/45 (0)	-
Salmonella spp	2/96(2.1)	0/10(0)	1/21(4.8)	1/14 (7.1)	2/45 (4.4)	0.364
Cryptosporidium spp	6/96(6.2)	1/10(10)	2/21(9.5)	1/14 (7.1)	4/45 (8.9)	0.828
ETEC*	34/96(35.4)	1/10(10)	4/21(19.0)	1/14 (7.1)	6/45 (13.3)	<0.001
STEC**	8/96(8.3)	0/39(0)	2/21(9.5)	0/14 (0)	2/45 (4.4)	0.063

*Significantly (P < 0.05) different between Diarrhea Group and Control Group.

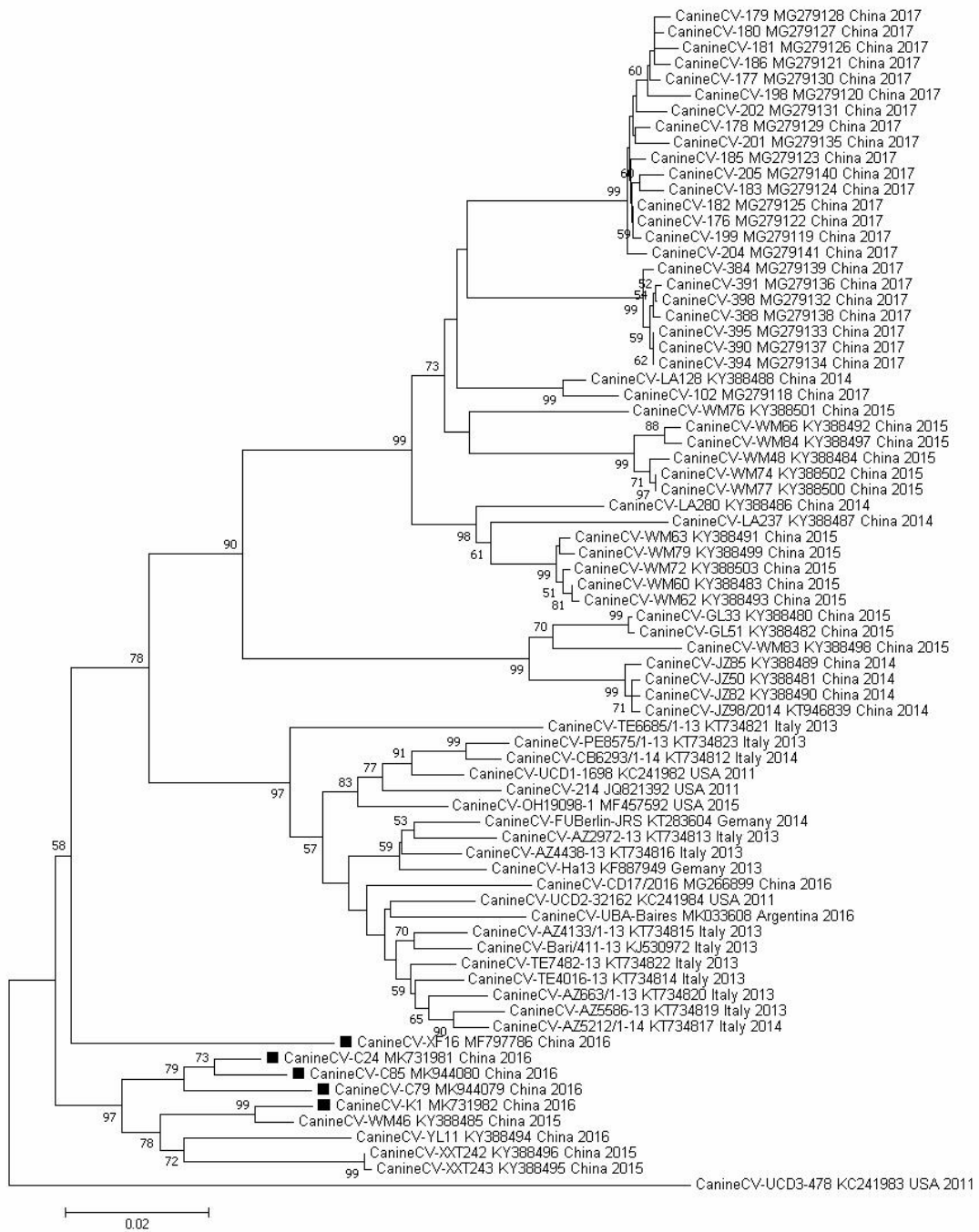
**Significantly (P < 0.1) different between Diarrhea Group and Control Group

Fig.S1 Phylogenetic analysis of canine circovirus and other circovirus (a) Neighbor-joining trees are based on the fulllength genome. **(b)** Neighbor-joining trees are based on the rep protein gene. **(c)** Neighbor-joining trees are based on the cap protein gene. For phylogenetic tree construction, the reference strains and GenBank accession numbers are as reported in Table 2. A statistical support was provided by bootstrapping over 1,000 replicates. The scale bars indicate the estimated numbers of nucleotide or amino acid substitutions. Filled circled reference strains first mentioned in this article.

(a)



(b)



(c)

